TIME AND DOSE DEPENDENTMODULATION OF THE BIOCHEMICAL AND MORPHOLOGICALMARKERS IN PESTICIDES EXPOSURE

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ABSTRACT

The spectrum of toxic effects induced by xenobiotics was evaluated from the serum and hepatic biochemical and morphological structural behaviour in pesticides acute, subchronically and chronically exposed rats. Male Wistar rats were intoxicated with Nevirol (N-phthalamic acid). The time evolution (24, 48, 72 hours) of serum and hepatic response induced by one dose exposure were compared with the cumulative effects obtained in short-time experiments (15 days) and in long-time (18 moths) chronic experiments. The serum enzymes: leucine aminopeptidase (LAP), ceruloplasmine (CER), glutamate dehydrogenase (Gl-DH), lactic dehydrogenase (LDH), were simultaneously investigated with the hepatic Ca^{2+}-, Mg^{2+} - and basal ATPases, LAP and Gl-DH. The protein-SH and nonprotein-SH groups, directly involved in the detoxification processes were also evaluated. The LAP level evolution may suggests a hepatocytes plasmamembrane disorder. A synthesis stimulation may be supported by unexpected high liver activity after 72 hours, that is not in a pozitive relationship with those released in the serum. Gl-DH exhibits the same seric and hepatic feature, so the total effect summarize the two activities. An alteration of hepatocyte energy metabolism is advocated by the feature of Ca^{2+}-, Mg^{2+}- basal ATPases. Protein- and glutathione-SH were early depleted, followed by higher values after 48 and in subchronically exposed rats. The hepatic structural injury supports good correlation with biochemical parameters and dose received.

INTRODUCTION

The release of any hepatic enzymes and factors into the serum is considered to be earlier and more sensitive indicators of hepatic damage than available changes in the morphological structure (4, 7).

A good informative model for assessing hepatotoxic effects following the chemical exposure consists in the simultaneous investigation of two areas: serum and tissue homogenate, completed by the morphological examination (4, 2).

The aim of this study was to compare any serum and hepatic enzyme values and to complete these data with the detoxification hepatic factors investigation in an acute and chronic experiment with Nevirol (N-phthalamic acid) - grow stimulating agent in vegetable and fruit, DL50 per os in rat, 3.000 mg/kg body.

The work includes the liver morphological investigation, too.

METHOD

Male Wistar rats weight 100-120 g approx. were divided into three lots: a control lot and two experimental lots.

Acute experiment:

- one dose - ½ LD50 (150mg) Nevirol received 6 male Wistar rats by gastric gavages;
- biochemical and morphological markers were investigated after 24h, 48h and 72h.

Chronic experiments:

- 15 doses - 1/10 LD50 (30mg/daily) Nevirol;
- 18 months – 500mg/kg food/daily Nevirol;
- Biochemical and morphological investigation of control and treated rats using current available methods.

Over the periods after dosing, the serum and hepatic homogenate bioenzymological factors of treated and control animals were investigated:

- serum and hepatic leucinaminopeptidase (LAP) (3);
- serum and hepatic glutamate-dehydrogenase (Gl-DH) (4, 6);

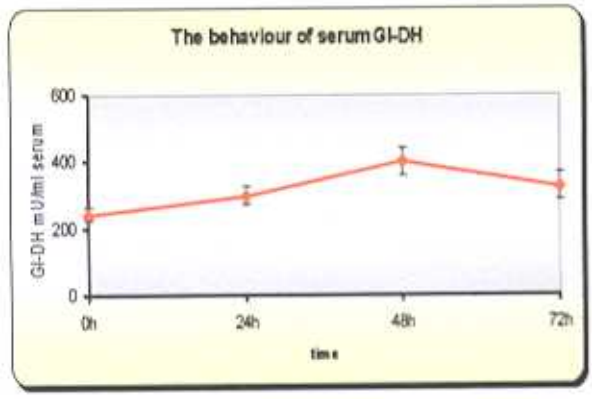
- hepatic basal Ca^{2+} and Mg^{2+} dependent ATPases (5, 4);
- hepatic protein- and nonprotein sulfhydryl groups (1).

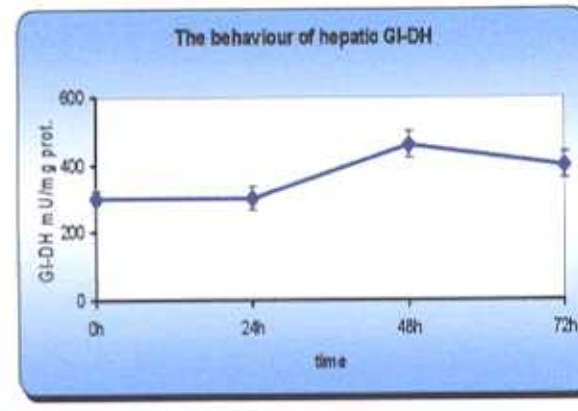
Histological assessments were performed over these periods (24, 48, 72 hours) on the hepatic fragments, by usual techniques: hematoxilin-eosin (HE), PAS (for glycogen).

RESULTS AND DISCUSSIONS

Gl-DH in acute exposure to Nevirol

- a similar pattern of the enzyme activity in serum and liver homogenate
- the peak of the two values at 48h exhibiting a high perturbation of the mitochondrial function.

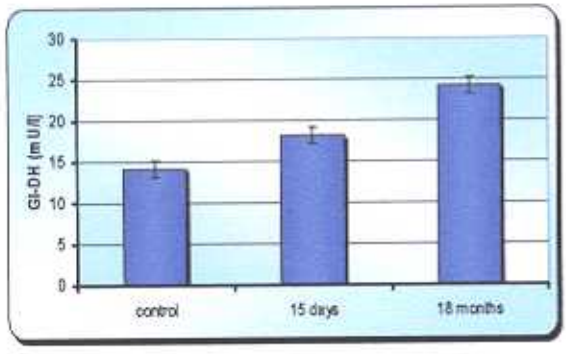


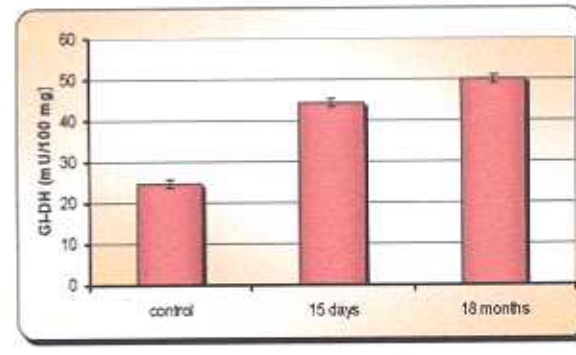


Short- and long-time chronic exposure

Cumulative effects:

- dose-effect pattern of the serum Gl-DH in short- and long-time exposure;
- slowly rate of Gl-DH magnitude in liver homogenate in long-time exposure.

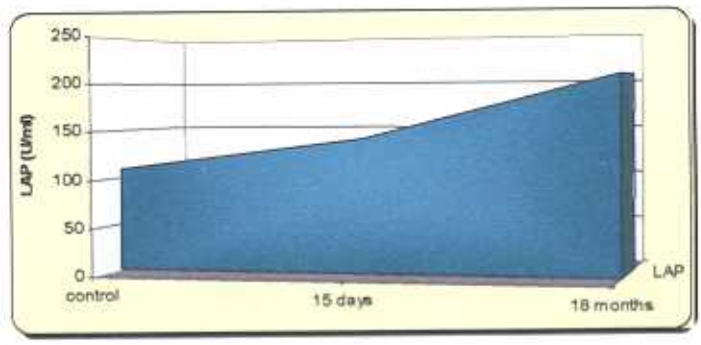




LAP in chronic exposure

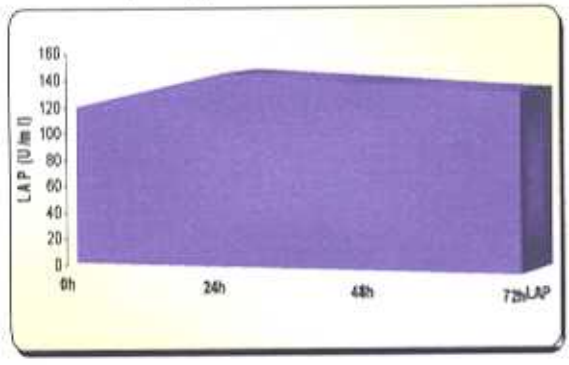
Cumulative effects:

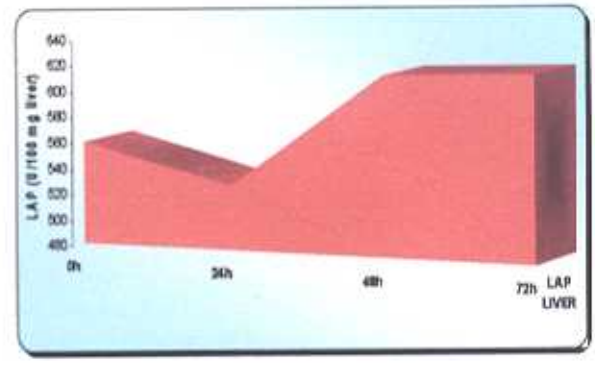
- LAP activity evolution in a dose-effect typed feature with
- higher perturbation of enzyme starting after a slow response to the first (15) doses of Nevirol.



Time evolution of the serum and hepatic LAP

- The serum enzyme values increased at 24 and 48 h, returning to control at 72 h is accompanied by a
- quite opposable feature in liver homogenate at the same time periods.

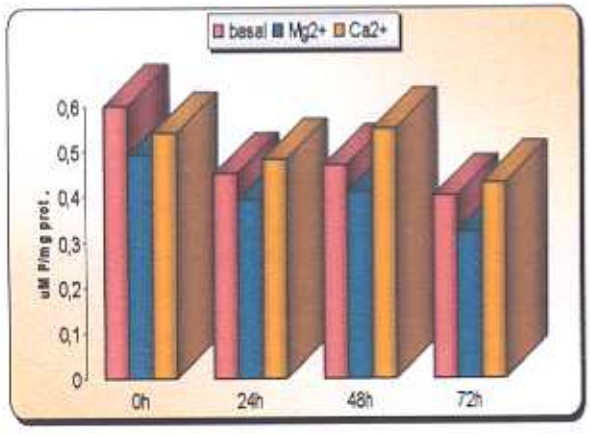


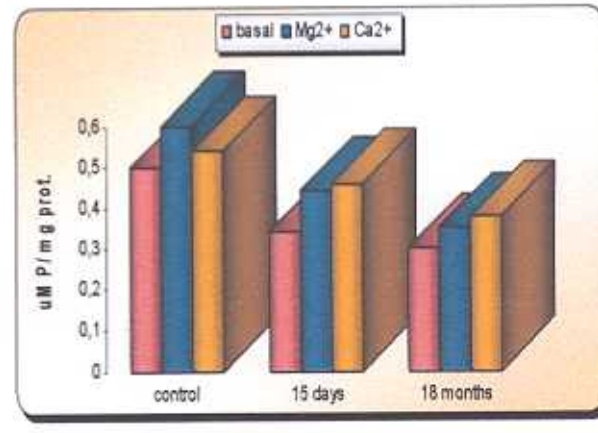


ATP-ases

The three ATP-ases activities followed

- similarly typed curves expressing a slight perturbation in acute dosing
- chronic exposure induces a dose-effect typed decreases of the ATP-ases activities.

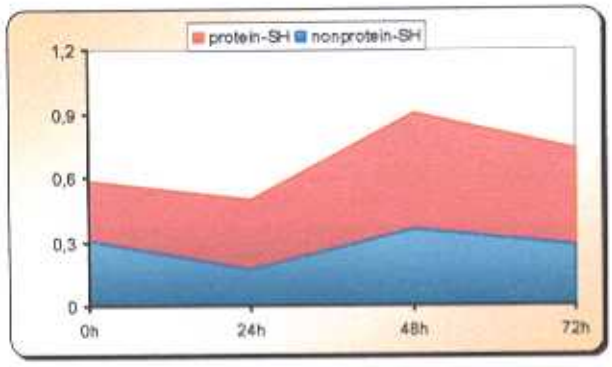


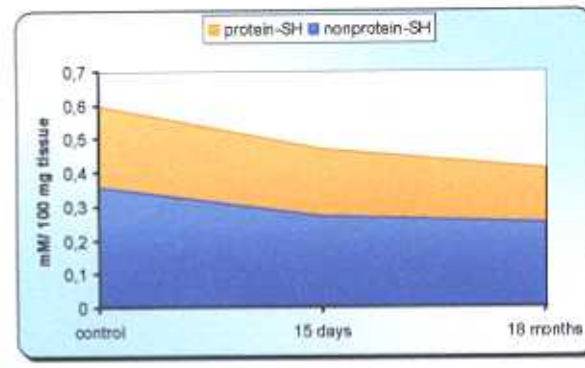


-SH

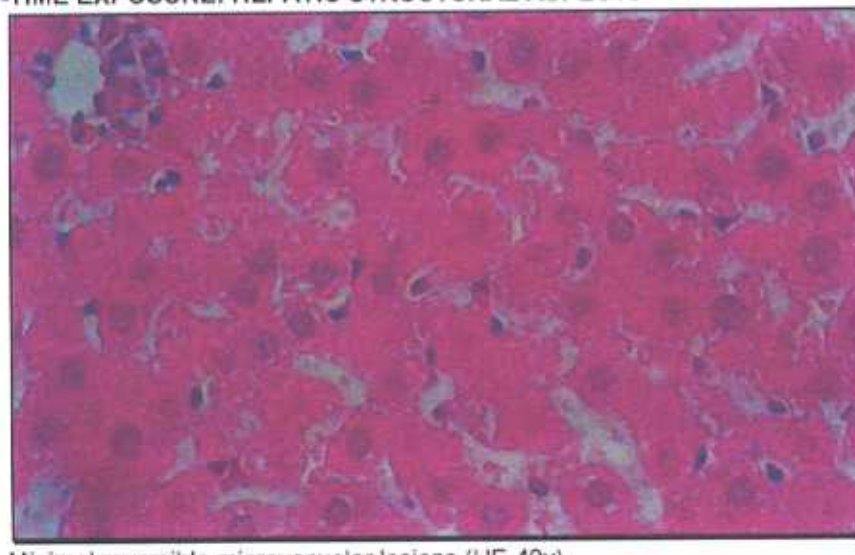
- -SH groups in hepatic homogenate showed a depletion during the first period after exposure, followed by a significant increase at 48 h and return to control at 72 h.

- Depletion induced by chronic exposure is appreciable for 15 doses, long time exposure giving low additionally perturbation.

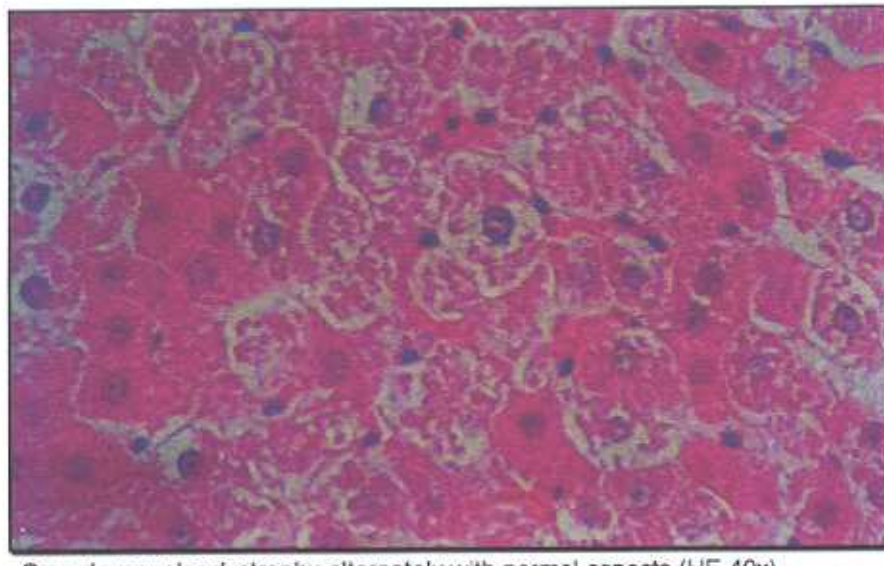




SHORT-TIME EXPOSURE: HEPATIC STRUCTURAL ASPECTS

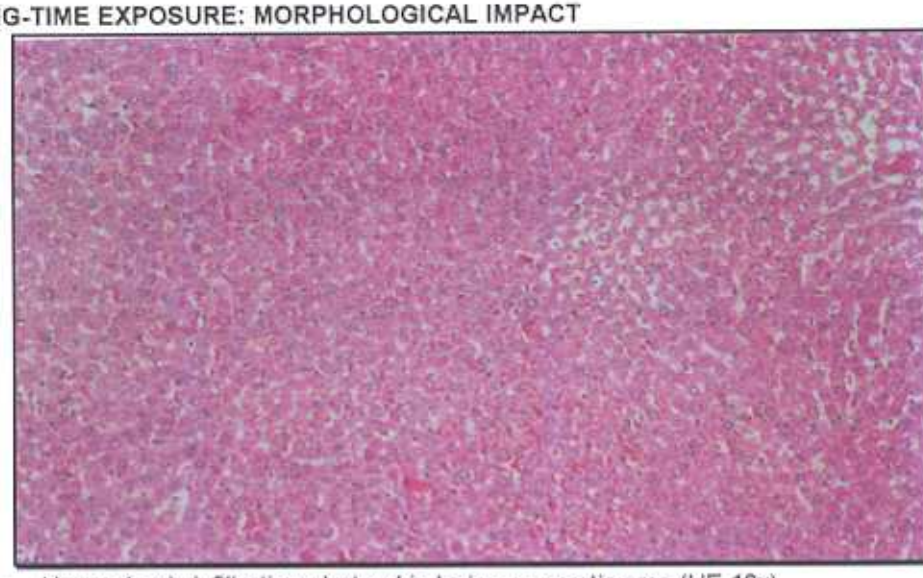


- Minimal reversible microvacuolar lesions (HE 40x)

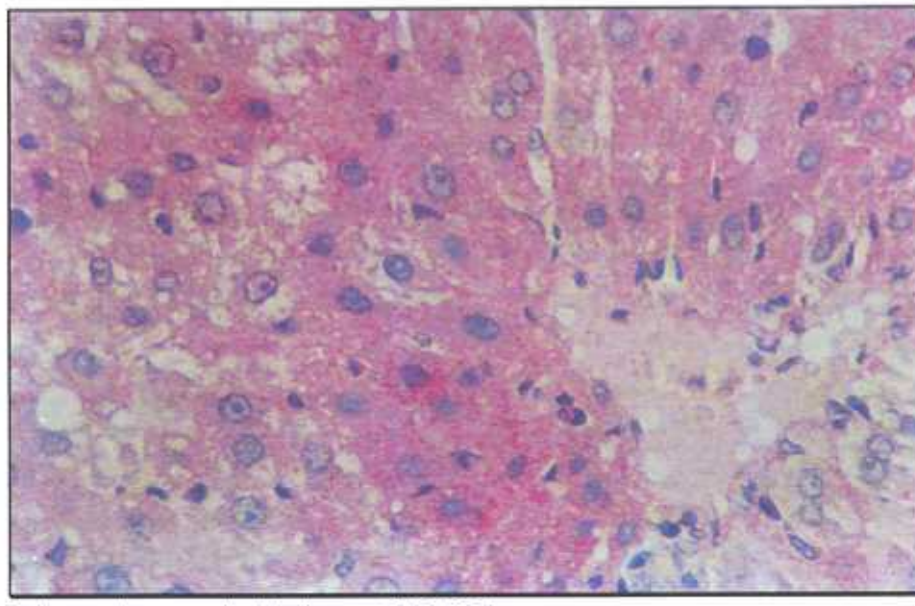


- Granulovacuolar dystrophy alternately with normal aspects (HE 40x)

LONG-TIME EXPOSURE: MORPHOLOGICAL IMPACT



- Hemorrhagic infiltration, dystrophic lesions, necrotic area (HE 10x)



- Large glycogen depleted area, (PAS 20x)

CONCLUSIONS

LAP - Plasma membrane disorder expressed by high serum activity at 24h, 48h, the overcharge of the liver at 72h; a dose-effect perturbation in persisting chronic cumulative dosing.

ATP-ases – Positive dose-effect reversible alteration of the active transmembranar ion transport and energy metabolism after one dose and severe perturbation in chronic repeated dosing, expressed by the depletion of Ca2+-, Mg2+ - and basal ATP-ases, statistically different from control.

GI-DH – Mitochondrial marker - exhibiting a high perturbation of the mitochondrial function in one-dose exposure and unexpected slowly rate additional perturbation in persisting exposure.

-SH groups - Detoxification /antioxidative indicators – were mainly time modulated exhibiting a depletion during the first period after exposure, a significant increase at 48 h and return to

control at 72 h; chronic exposure induced an appreciable depletion for 15 doses, persisting exposure giving lowly additional perturbation.

Morphological aspects – consist a structural support good correlated with the biochemical markers behavior.

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